

Attorney's Docket No.: 56446-20001.20/-002006/D1100-4US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Short et al. (as amended)
Serial No. : 09/884,889
Filed : June 19, 2001
Title : CATALASES

Art Unit : 1652
Examiner : Rebecca E. Prouty, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

OFFICIAL

RECEIVED
CENTRAL FAX CENTER
APR 22 2004

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as a CEO and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

2. I declare that procedures for modifying nucleic acids were conventional and routine in the art at the time of the invention. One of ordinary skill in the art using the teaching of the specification would have been able to make a catalase-encoding nucleic acid having at least 65% sequence identity to SEQ ID NO:5 or SEQ ID NO:7, or a catalase-encoding nucleic acid comprising at least 30 or 35 or more consecutive nucleotides of a sequence having at least 65% sequence identity to SEQ ID NO:5 or SEQ ID NO:7, or a catalase-encoding nucleic acid that hybridizes under the defined stringent hybridization conditions, to practice the methods of the invention without undue experimentation. It was considered routine by one skilled in the art at the time of the invention to screen for multiple substitutions or multiple modifications in a nucleic acid sequence for functional variations, including screening for a genus of catalase-encoding nucleic acids. For example, high through-put methods for screening for enzyme

Applicant : Short et al. (as amended)
Serial No. : 09/884,889
Filed : June 19, 2001
Page : 2 of 4

Attorney's Docket No.: 56446-20001.20/-
002006/D1100-4US

activity, such as catalase activity, were well known in the art. While the numbers of samples needed to be screened may have been high, the screening procedures were routine and successful results (e.g., finding a genus of nucleic acids encoding catalases) predictable. At the time of the invention it would have been considered routine by one skilled in the art to generate and screen multiple substitutions or multiple modifications in an exemplary nucleic acid sequence and predictably generate a genus of nucleic acids encoding catalases.

3. I declare that it was not necessary for the skilled artisan to understand which specific regions of catalase structure may be modified without affecting function or activity, or, which specific regions of catalase structure should be modified to generate altered enzyme activity, to practice the methods of the invention because methods for modifying sequences, generating catalase-encoding sequences and screening for activity at the time of the invention were routine and predictable. Methods for sequence modifications were sufficiently routine and predictable at the time of the invention to predictably generate catalase-encoding sequences without need of knowing which specific regions of catalase structure affect catalase function or activity. For example, on pages 30 to 33, the specification gives a detailed description of an exemplary method for sequence modification called Gene Site Saturation MutagenesisTM (GSSMTM). In one aspect of GSSMTM, degenerate oligonucleotides comprising degenerate N,N,N cassettes can be used for subjecting each original codon in a parental polynucleotide template to a full range of codon substitutions. Thus, GSSMTM allows for mutagenizing each and every amino acid position in a parental polypeptide to generate amino acid changes that can be routinely screened for their effect on activity. Another exemplary method for sequence modification, called synthetic gene reassembly, or SLR, is described on pages 13 to 15 of the specification. As noted in the specification on page 15, lines 15 to 24, SLR allows a systematic examination and screening procedure to be performed, allowing a potentially very large number of progeny molecules to be examined systematically in smaller groups. Methods known at the time of the invention for modifying nucleic acid sequences, such as GSSMTM, SLR, or the other methods described in the specification, in combination with high through-put enzyme activity screening known at the time of the invention, made methods that require previous knowledge of protein tertiary structure, active sites and the like obsolete and unnecessary. Accordingly, using

Applicant : Short et al. (as amended)
Serial No. : 09/884,889
Filed : June 19, 2001
Page : 3 of 4

Attorney's Docket No.: 56446-20001.20/-
002006/D1100-4US

methods known in the art at the time of the invention, e.g., GSSM™ or SLR, it would not have been necessary to understand which specific regions of catalase structure needed to be modified to generate the genus of nucleic acids for practicing the methods of the invention.

4. I declare that the specification provides sufficient guidance to one of ordinary skill in the art as to whether a nucleic acid falls within the scope of the genus used in the claimed methods. Methods for determining the requisite structure (sequence based on percent sequence identity to an exemplary nucleic acid) and function (catalase activity) are clearly set forth in the specification. Dr. Short declares that at the time of the invention, high through-put *in vivo* (e.g., whole cell) nucleic acid expression and enzyme activity screening protocols were well known in the art. The specification sets forth an exemplary catalase screening assay to determine if a nucleic acid is within the scope of the genus used in the claimed methods, *inter alia*, on pages 72 to 73, Example 2. Methods for determining sequence identity were also routine and well known in the art at the time of the invention. The specification describes methods for determining whether a nucleic acid has a percent sequence identity to an exemplary polynucleotide on, *inter alia*, pages 55 to 70 of the specification. All of these protocols were routine in the art at the time of the invention and positive results (e.g., determining if a nucleic acid is within the scope of the genus used in the claimed methods, e.g., a catalase-encoding nucleic acid at least 30 consecutive nucleotides of a sequence having at least 65% identity to SEQ ID NO:5 or SEQ ID NO:7) predictable.

4. I declare that methods of making and screening procedures used to identify nucleic acids used in the claimed methods (e.g., identifying the genus of catalase-encoding nucleic acids) were all well known in the art and at the time this application was filed. All were routine protocols for the skilled artisan. While the numbers of alternative species that needed to be screened may have been high, the protocols for screening were routine and positive results predictable. The specification provided sufficient guidance to one of ordinary skill in the art to make and use the described genus of nucleic acid to practice the claimed methods. Thus, the skilled artisan using Applicants' written disclosure could have practiced the instant claimed invention without undue experimentation.

Applicant : Short et al. (as amended)
Serial No. : 09/884,889
Filed : June 19, 2001
Page : 4 of 4

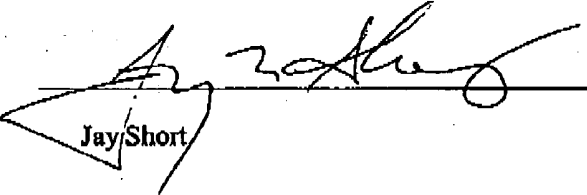
Attorney's Docket No.: 56446-20001.20/-
002006/D1100-4US

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: _____

4/21/04


Jay Short